user manual
proteomics

Ettan *Spot Picker*

User Manual

um 18-1147-61

amersham pharmacia biotech

Important user information

All users must read this entire manual to fully understand the safe use of Ettan™ Spot Picker.

WARNING!

The Warning sign highlights an instruction that must be strictly followed in order to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Caution!

The Caution sign is used to call attention to instructions or conditions that must be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Declaration of conformity

Safety standards

This product meets the requirements of the Low Voltage Directive 72/23/EEC through the harmonized standard EN 61 010-1:1993 + A2:1995.

EMC standards

This product meets the requirements of the EMC Directive 89/336/EEC through the harmonized standards EN 50081-1 and EN 50082-1.

WARNING!

This is a Class A product. In a domestic environment, this product may cause radio interference, in which case the user may be required to take adequate measures.

The **CE** symbol and corresponding declaration of conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked Amersham Pharmacia Biotech instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Pharmacia Biotech except for alterations described in this manual.

Office Addresses

Amersham Pharmacia Biotech AB
SE-751 84 Uppsala
Sweden

Amersham Pharmacia Biotech UK Limited
Amersham Place Little Chalfont
Buckinghamshire
England HP7 9NA

Amersham Pharmacia Biotech Inc.
800 Centennial Avenue
P.O. Box 1327 Piscataway N.J. 08855-1327
USA

Amersham Pharmacia Biotech Europe GmbH
Munzinger Strasse 9
D-79111 Freiburg
Germany

Amersham Pharmacia Biotech KK
Sanken Building
3-25-1 Hyakunincho, Shinjuku-ku
Tokyo
Japan

Contents

4 Selecting spots on the gel and creating a pick list

5 Setting up the Spot Picker

6 Picking a gel

List of figures

1 Introduction

The Ettan™ Spot Picker is designed to automatically excise polyacrylamide gel plugs, containing proteins from 2D gels, and then transfer the plugs to microplates. These gel plugs are suitable for further processing prior to MALDI-TOF mass spectrometry analysis.

The Spot Picker is compatible with 2D gels that have been post-stained with Coomassie™, silver or SYPRO™ dyes, and for pre-labelled proteins. Gels must be immobilized on plastic backing or glass.

Spots of interest can be evaluated and selected by using the ImageMaster™ 2D Elite gel image analysis software. A pair of reference markers must be included within the gel and also detected during image analysis. These reference markers are used to enable the Spot Picker to transform image (pixel) X-Y co-ordinates for each spot into a millimetre position to pick from.

Following excision from the gel, the plugs are dispensed into 96-well microplates, along with a volume of dispensing fluid. The Ettan Spot Picker is capable of picking up to 9600 plugs in any single picking run.

1.1 The Ettan Spot Picker system

The Ettan Spot Picker system comprises the following:

- Ettan Spot Picker instrument
- Ettan Spot Picker Instrument Control Software, running under Windows NT operating system on a PC
- PC with frame grabber card for video display

Fig. 1-1 shows the Ettan Spot Picker system.

A detailed description of the Ettan Spot Picker instrument is provided in the *Ettan Spot Picker Instrument Handbook*.

Fig 1-1. The Ettan Spot Picker system.

1.2 Method overview

With the Ettan Spot Picker, gel plug picking can be defined in a number of steps:

1 Preparing and pouring the gel onto a backing, including reference markers. Alternatively, use precast gels and include the reference markers before scanning.

2 Running the 2D separation.

3 Fixing and staining the gel after electrophoresis.

4 Scanning the gel including applied reference markers.

5 Analysis of the gel image, selecting the protein spots to pick and creating the pick list file.

6 Loading the gel onto the Ettan Spot Picker and excising the selected gel plugs into microplates.

1.3 The User Manual

The *Ettan Spot Picker User Manual* provides technical information and operating instructions for the Ettan Spot Picker instrument and the Ettan Spot Picker Instrument Control Software. Gel casting and gel running are described briefly. In addition, instructions for scanners and gel image evaluation softwares are included. For detailed information on the instrument, the user is referred to the *Ettan Spot Picker Instrument Handbook*.

1.4 Accessories

In addition to the items delivered with the system, Ettan Spot Picker requires the following accessories:

- Gel scanner: ImageScanner™ or Typhoon™
- Gel image evaluation software: ImageMaster 2D Elite
- 96-well microplates

See the *Ettan Spot Picker Instrument Handbook* for ordering information.

1.5 Associated documentation

The *Ettan Spot Picker Instrument Handbook* provides safety instructions, technical information and basic operating instructions for the Ettan Spot Picker instrument. In addition, maintenance schedules and instructions for user maintenance are included.

2 Safety instructions

IMPORTANT! To avoid any risk of injury, the instrument should only be operated by properly trained personnel and always in accordance with the instructions provided.

The purpose of this chapter is to describe safety precautions and present all safety labels that are attached to the instrument. Built-in safety functions are described, and emergency and disposal procedures are included.

2.1 Safety precautions

1 Read this entire manual before using the Ettan Spot Picker instrument.

2 This instrument is designed for indoor use only.

3 The instrument must always be used with the protective earth lead of the power cord correctly grounded to earth at the mains outlet.

4 To permit sufficient cooling, ensure that the vents at the top and bottom of the instrument are not covered.

5 Do not operate the instrument in extreme humidity (above 95%). Avoid condensation by letting the unit equilibrate to ambient temperature.

6 Do not operate the instrument in direct sunlight.

7 Keep the instrument dry and clean. Wipe regularly with a soft damp tissue. Let the instrument dry completely before use.

8 Any equipment connected to the instrument should meet the requirements of the EN 61 010-1 or other international safety standards.

WARNING! Do not use the Ettan Spot Picker in any other way than described in this User Manual. The protection provided by the instrument may be weakened if other operations are performed.

2.2 Safety labels

The following safety labels are attached to the Ettan Spot Picker instrument to warn the user of potentially hazardous conditions:

At the picker head

Fig 2-1. Safety label located on the Y-arm, near the picker head.

WARNING! MOVING PARTS. The picker head and camera assembly can make sudden, rapid movements. Keep all body parts clear when the Spot Picker is in operation.

WARNING! Never put your hands underneath the picker head during instrument operation. The picker head has the capacity to puncture skin. In the event of an emergency, press the **Stop** button on the Spot Picker: the instrument immediately ceases movement and all motors are de-energized.

WARNING! To prevent fire or shock hazard, do not spill liquids into the camera body.

2.3 Other warnings

WARNING! HEAVY OBJECT. The Ettan Spot Picker weighs about 40 kg. Two persons are required to lift the instrument.

WARNING! NO SERVICEABLE PARTS INSIDE. Do not open covers. Service and maintenance should be performed by qualified personnel.

2.4 Emergency procedures

2.4.1 Emergency shutdown

In the event of an emergency, press the **Stop** button on the Ettan Spot Picker front panel. The instrument immediately ceases movement and all motors are de-energized.

2.4.2 Power failure routine

In the event of a power failure, the experiment is interrupted in an undefined state. However, the data for gel plugs picked up to the power interruption is saved.

When power returns, the following will happen:

1 The picker head and the syringe pump (dilutor) are homed.

2 The PC starts and displays the log-in dialogue.

2.4.3 Restart Procedure

In the event of system shutdown due to power failure, emergency stop or process interruption, malfunctions must be rectified before the Ettan Spot Picker is restarted.

To restart the Ettan Spot Picker:

1 Check that the **Power** indicator on the Ettan Spot Picker instrument lights.

2 Select **Programs:Ettan Spot Picker:Ettan Spot Picker** from the Windows **Start** menu.

Reload the resultfile and continue picking, see instructions in Section 6.10. The last picked gel plug may be lost. This depends on the exact moment when the interruption occurred.

3 Preparing the gel

3.1 Introduction

Spot picking with the Ettan Spot Picker requires that gels are precast on backing (e.g. Ettan DALT II Pre-cast Gel 12.5) or immobilized on backing during casting. Two different types of backing may be used, either: GelBond™ PAG film; or a glass plate, treated with a Bind-Silane solution.

Note: *To scan a gel with fluorescently labelled proteins, it is important that GelBond is not used as the gel backing. The reason for this is that GelBond is a plastic material and all plastics have intense fluorescenses at the wavelengths used for scanning.*

3.2 Bind-Silane treating glass plates

The following protocol for treatment of glass plates was optimized for Bind-Silane from Amersham Pharmacia Biotech.

Note: *It is important that glass plates are properly clean. Before re-use, soak the plates overnight in a 5% Decon^{TM} 90 solution. Do not leave plates standing in a Decon solution for a longer time as this will eventually cause etching due to the alkali nature of Decon.*

1 Thoroughly wash the plate to be treated. Take care to remove any gel fragments attached to the plate from previous gels. The careful cleaning of the glass plates before casting is important, to ensure a uniform coating with the Bind-Silane and, to avoid keratin contamination.

2 Thoroughly rinse the plates with ddH_2O to remove Decon

3 Dry the plate using a lint-free tissue or leave them to air dry.

4 Prepare the Bind-Silane working solution, see Table 3-1 :

Reagent	Quantity
Ethanol	8 ml
Glacial Acetic acid	200 µl
Bind-Silane	10 µl
Double distilled H_2O	1.8 ml

Table 3-1. Bind-Silane working solution.

5 Pipette 2-4 ml (depending on plate size) of the Bind-Silane solution onto the plate and distribute equally over the plate with a lint-free tissue. Cover the plate to prevent dust contamination and leave to air dry on the bench for 1-1.5 hours

6 Polish the plate with a lint-free tissue, moistened with a small amount of double-distilled water or ethanol.

The gels will stay attached to the glass during electrophoresis, staining procedures, scanning and storage.

3.3 Positioning of the reference markers

Spot picking with the Ettan Spot Picker requires the use of reference markers. The reference markers are used to enable the Spot Picker to transform image (pixel) X-Y co-ordinates for each spot into a millimetre position to pick from. This enables picking from gels where the protein spots are fluorescently labelled, as well as from gels where the proteins have been stained with a visible stain.

The reference markers are designed so that they will be visible on the scanned image of a gel (fluorescent or visible protein stain) as well as being visible to the marker detection camera on the Ettan Spot Picker. Two reference markers must be included in each gel that is to be picked from.

The reference markers are self-adhesive and should be attached to the gel backing (glass plate or GelBond film) prior to gel casting, or at the latest, before the scanning of the gel. The reference markers can be used in both densitometric and fluorescence scanning.

3.3.1 Positioning before gel casting

It is important that the markers are appropriately placed on the gel backing. The markers should be placed according to the following protocol. Take care not to place the markers where they will interfere with the pattern of protein spots in the gel.

1 Place the spacers on the edge of the treated glass plate/GelBond, to ensure that the spacers do not cover the markers.

2 Place the marker approximately half-way along this edge, away from the spacer, but not so far as to interfere with the protein spot pattern.

3 Repeat steps 1 and 2 for the other edge of the gel backing.

4 When finished, the markers should be in positions similar to those shown in Fig. 3-1.

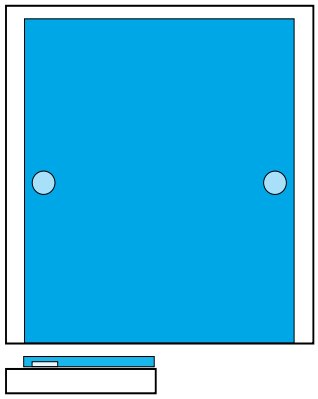


Fig 3-1. Position of the reference markers on the gel backing when gel has been cast.

3.3.2 Positioning before gel scanning

If there is a sufficient backing outside the gel area, for example Hoefer™ SE 600 gels, then it is possible to attach the reference markers, after casting just before scanning.

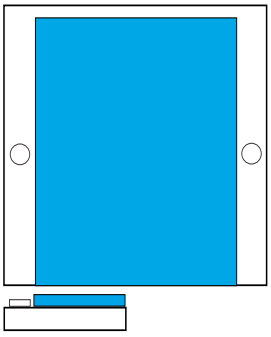


Fig 3-2. Position of the reference markers on the gel backing outside the gel.

If there is not a sufficient backing-area outside the gel, it is possible to attach the reference markers below the backing before gel scanning. This is the recommended procedure for Ettan DALT II Pre-cast Gel 12.5.

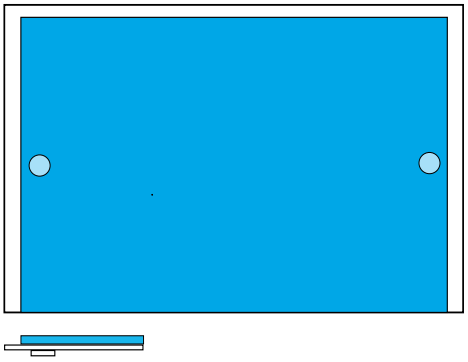


Fig 3-3. Position of the reference markers below the gel backing.

Note: *When attaching the reference markers on a gel backing after the gel casting or electrophoresis run, the surface of the backing must be completely dry, to ensure good adhesion of the markers.*

3.4 Gel casting

Now assemble the gel holder for casting as described in the manufacturers instructions. Pour the gels as soon as they are correctly assembled.

3.5 Gel running conditions

The gel being attached to a backing will not affect the standard gel running conditions for a given sample. However, it is important that the Immobiline™ DryStrip is correctly orientated. The Immobiline Dry strip should be placed as normal (e.g. acidic end to the left), with the gel backing lower-most.

3.6 Gel staining

To find examples of fixing and staining protocols, see Chapter 9 Staining protocols.

3.7 Gel scanning with the ImageScanner

3.7.1 Gel position

In order to secure the targeting accuracy of the Ettan Spot Picker it is of particular importance to place the markers in the correct position during scanning.

- The markers must be placed in the middle of the scanning area (Fig. 3-4).
- The top of the gel, which was in contact with the Immobiline Dry strip, must be placed on the right side when looking at the scanner as showed in Fig. 3-4.
- The gels must be scanned upside-down, with the gel in contact with the glass surface of the scanner, with a thin layer of water or gel storage liquid between. The liquid layer will also make it easier to lift the gel after scanning.

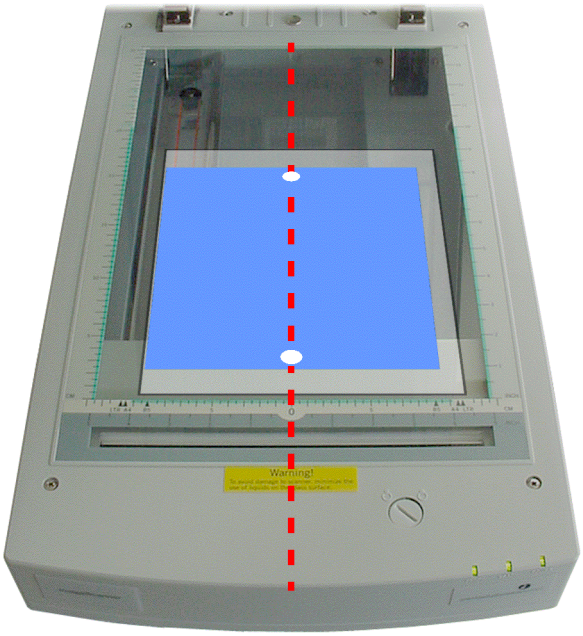


Fig 3-4. Correct positioning of the gel on the ImageScanner.

1 Pour a few ml of gel storage liquid, or water, onto the glass surface of the scanner.

2 Hold the gel (on backing) at an angle over the scanning surface.

3 To avoid air bubbles, slowly lower the gel until it is flat and resting on the scanning surface.

4 To avoid seeing water drops on the gel image, wipe any drops off from the upper surface of the gel backing with a lint-free tissue.

3.7.2 Scanning

1 In the ImageMaster Labscan software, select the function **Scan a new image.**

2 Select **Refresh preview.**

3 Select the scan area with the mouse arrow. Start in the lower left position of the gel image on the screen. Drag an area to the upper right position of the preview area, even if this area is not showing the gel.

Note: *It is crucial to maximize the selected scan area in the upper right position, before releasing the mouse button. Otherwise, the calibration of the ImageScanner will be affected, and resulting in imprecise spot picking.*

4 Select the resolution: 300 dpi and Scan mode: transmissive.

5 Start scan.

6 After scanning, press the **Rotation tool** button, and rotate the image counter clockwise, once. The resulting image will show the gel with the acidic pH to the left, basic pH to the right and low Mw at the lower end.

7 Save the image as a .tif file with **File:Save as.**

Note: *To secure the picking accuracy of the Spot Picker, no other manipulations with the gel image is allowed (e.g. rotating, or resizing).*

3.8 Gel scanning with the Typhoon

It is important to place the gel directly onto the scanner plate. Do not reassemble.

The gel must be scanned upside-down, with the gel in contact with the glass surface of the scanner, with a thin layer of water or gel storage liquid between. The liquid layer will also make it easier to lift the gel after scanning.

1 Pour a few ml of gel storage liquid, or water, onto the glass surface of the scanner.

2 Hold the gel (on backing) at an angle over the scanning surface.

3 To avoid air bubbles, slowly lower the gel until it is flat and resting on the scanning surface.

3.8.1 SYPRO ruby stained gels

It is recommended to use the green laser (532 nm), and the emission filter of 610 nm with 30 nm bandpass. Use the platen focus setting.

3.8.2 SYPRO orange stained gels

The green laser (532 nm) is recommended. The emission filter of 580 nm with 30 nm bandpass should be selected. Use the platen focus setting.

3.8.3 Image on screen after scanning

Before saving the image, the image on the screen has to be in the orientation with the acidic pH to the left, basic pH to the right and low Mw at the lower end.

Rotate the image to the specified position and save the image.

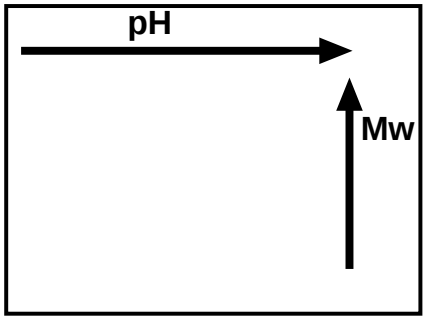


Fig 3-5. Orientation of gel image before saving.

4 Selecting spots on the gel and creating a pick list

4.1 Using ImageMaster 2D Elite

The basis of spot picking with the Ettan Spot Picker is a pick list created by an image analysis software. This section describes how to create this pick list, using ImageMaster 2D Elite (V3.00 and higher).

Essentially, the pick list needs to contain the location, in pixels, of the centre of each spot you wish to pick. It must also contain the pixel co-ordinates of the centres of the two reference markers.

This section assumes that the user is familiar with at least basic spot detection and editing using ImageMaster. For more detailed information on the use of ImageMaster, consult the *User Manual*.

4.1.1 Simulating the picker head size

When a spot boundary has been detected by ImageMaster or drawn by the user, it is assigned a position in the image in terms of X and Y pixel co-ordinates. This corresponds to the centroid of the spot (as defined by the spot boundary). These are the co-ordinates used to define the position that the picker head will remove a gel plug from. It is therefore very important that spots are accurately detected so that the correct area of gel is picked.

The following points should be considered during spot selection for picking:

- The dimension of the picker head limits picking of spots very close to each other. It is advisable to get an idea of the size of the picker head in the gel image by using a pen tool of appropriate size (Fig. 4-1).

- It may be preferable to manually specify the picking position for spots with irregular or long drawn-out shapes and for very large spots.

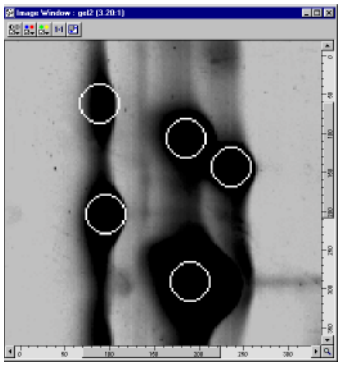


Fig 4-1. The use of Draw Spots pen tool to simulate the size of the picker head.

By using the **Draw Spots** tool, an approximate size of the picker head on the scanned image can be seen. For a gel image which was scanned at 300 dpi, a pen size of 17 will represent a 1.4 mm diameter picker head. Similarly, for a gel image scanned at 300 dpi, pen size 24 corresponds to a 2.0 mm picker head.

4.1.2 Detection and editing of the markers

The two reference markers included in the gel must also be detected as spots. Determining the centre of the marker is very important for accurate picking. Often, the reference markers are not accurately detected with the parameters used for detecting the protein spots in your image. If the reference markers have not been correctly detected, then follow these steps to define the reference markers.

Alternative 1

- Use the **Grow Peaks** tool and click inside the reference marker.

Alternative 2

1 Select a pen size slightly smaller than the size of the reference marker.

2 Draw a spot on the reference marker, placing it centrally on the marker.

3 Use the **Grow Edge** tool to fit the spot boundary to the boundary of the marker.

Note: *For accurate spot picking, it is essential that the reference markers are correctly detected. When the edges of the markers are not properly detected, the position on the pick list given for the centre of the marker will be incorrect. This will lead to an incorrect transformation of the spot co-ordinates from the pick list into mm positions for the picker to pick from.*

Make sure that the position-reference markers were detected as distinct, circular spots and that the outline of the spot well corresponds to the outline of the marker. This is illustrated in Fig. 4-2.

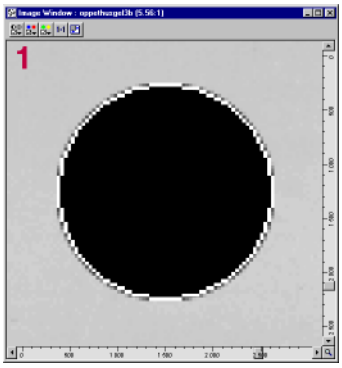


Fig 4-2. Correctly detected reference marker.

Drops of water below and above the gel during the scanning can affect the detection as seen in Fig. 4-3, 2.

An imprecise use of the detection tools can also result in an incorrect detection, see Fig. 4-3, 3

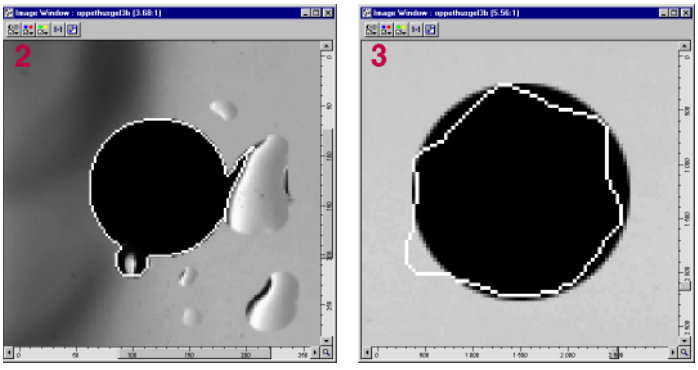


Fig 4-3. Incorrectly detected reference markers.

4.1.3 Detection and selection of spots for picking

After performing spot detection and spot editing, ImageMaster generates a measurements table containing spot related data.

When the measurements table is saved as a pick list, all the spots in the table will be picked. Therefore, if only a selection of the spots are requested to be picked, they have to be defined. This can either be done manually or by using the spot filtering tools.

Manual selection of spots to be picked

Once the spot editing is satisfactory, go to the spot filtering mode.

1 Deselect all spots by pressing the **Select/Deselect all** button. All the spots should appear blue. If they do not, press the button again.

2 Select the spots for picking by clicking on them with the left mouse button.

3 **The reference markers must also be selected.**

4 In case of errors, click on the spot with the right mouse button to deselect the spot.

5 Once this process is finished, see Section 4.1.5 Creating the pick list.

Automated selection of spots to be picked

Once the spot editing and spot matching are satisfactory (they must be complete to apply the automatic filtering), go to the spot filtering mode.

Decide to apply either *comparison* or *range* parameters. If parameters are set in both *comparison* and *range*, they will both be applied when the **Apply Filter** button is pressed, no matter which window is displayed.

- The *Range* parameters specify explicit ranges of values that a spot must have in its measurement fields in order to be selected.
- The *Comparisons* parameters select spots based on comparisons with spots that are matched to it in other gels.

For a detailed explanation of how to use these functions, please refer to the *ImageMaster reference manual* and/or help function.

After the automatic filter has been applied, the reference markers have to be selected manually if they have not been automatically selected. At this stage, it possible to manually edit the spot selections, as described in the preceding section.

Once this process is finished, see Section 4.1.5 Creating the pick list.

4.1.4 Designating the reference markers

The reference markers must be distinguished from protein spots in the pick list. This is achieved in ImageMaster by putting an entry into the *comment* field of the reference markers.

1 In the main image window, select **Edit:Edit Spot Fields**.

2 Select the arrow tool, and click on the left hand side reference marker.

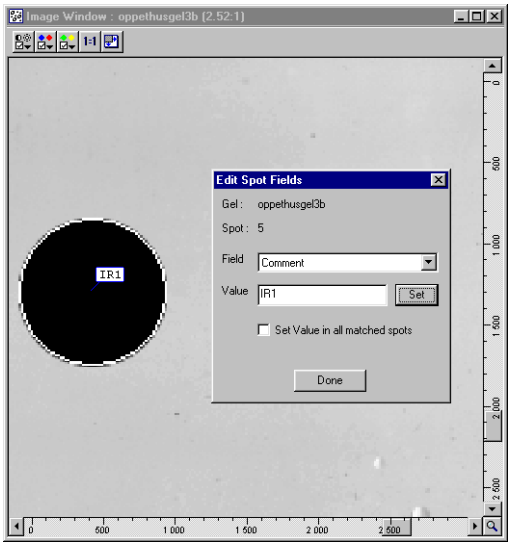
3 In the **Edit Spot Fields** dialogue box, select the **Field** as comment.

4 Enter "**IR1**"(uppercase letters, with no space between) into the **Value** field and press **Set**. This designates that the co-ordinates from this spot represent the first reference marker.

5 With the selected arrow tool, click on the right hand side reference marker.

6 Enter "**IR2**" into the **Value** field and press **Set**.

7 Press **Done**.



8 To view these comments on screen, go to **Tools:Options,** select the **Display** tab and tick the **Data annotations** box and set the field to **Comment.**

9 Check that "IR1" and "IR2" are in the **Comment** column in the **Measurements window.**

Note: When using ImageScanner, it is strongly recommended that reference markers are assigned in the manner described above. This way, reference marker 1 will always be on the left-hand side of the gel in the geltray (if the Immobiline Dry strip is at the top) and marker 2 will always be on the right. This will greatly aid identification of the different markers.

4.1.5 Creating the pick list

The pick list is created from the measurements window. However, before the pick list can be saved, the measurements table must be put in the correct format, as described below. If the pick list is not saved in this format, the picker will not recognise the file and will, therefore, not be able to pick the spots.

To create the pick list in the correct format:

1 Select **Tools:Options**. When the options window appears, select the **Display** tab. Set the **Co-ordinate** field to **Pixels**. Click **OK.**

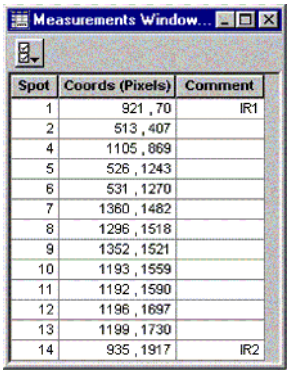
2 Activate the **Measurements Window.**

3 Using the **Select Fields** button, make sure that only the **Spot, Scaled Coords (Pixels)** and **Comment** fields are selected.

4 Deselect any other column by un-ticking the appropriate box.

5 If the order of the columns (left to right) does not appear the same as in Fig. 4-4, use the **Move Up** and **Move Down** buttons in the field selection box until they are correctly ordered.

6 The position of the reference markers (spots 1 & 14 in Fig. 4-4) in the pick list is unimportant.



Spot	Coords (Pixels)	Comment
1	921 , 70	IR1
2	513 , 407	
4	1105 , 869	
5	526 , 1243	
6	531 , 1270	
7	1360 , 1482	
8	1296 , 1518	
9	1352 , 1521	
10	1193 , 1559	
11	1192 , 1590	
12	1196 , 1697	
13	1199 , 1730	
14	935 , 1917	IR2

Fig 4-4. Correct appearance of the Measurements Window.

4.1.6 Saving the pick list file

When the measurement window table is correctly formatted, the pick list can be saved.

1 With the **Measurements Window** active, select **Edit:Copy To File**.

2 Enter a suitable file name for the pick list.

3 The pick list must be saved as a text (*.txt) file.

No further editing of the pick list should be done at this point.

If any errors are found in the pick list, it is preferable to go back to the appropriate step and create a new pick list.

4.2 Creating pick list files from alternative gel image evaluation softwares

Ettan Spot Picker Instrument Control Software can not handle original pick list data from alternative 2D gel image evaluation softwares. However, if the required data can be exported to Microsoft Excel™, it will be possible to use the data for spot picking with Ettan Spot Picker.

To create a pick list from an alternative 2D gel image evaluation software:

1 Export the data to Microsoft Excel. The following data are required:

- Spot identity
- The co-ordinates of the centre of the spots
- The co-ordinates must be in pixels
- The x and y co-ordinates must have separate columns

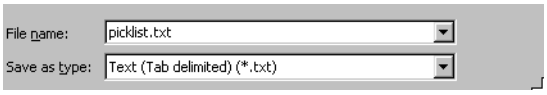
2 Edit the data in Excel to create a table in a correct format:

- The table must contain only four columns
- The headings of the columns must be as follow: id, x, y, comment
- The comment column must contain the IR1 and IR2 annotations for the reference markers. If the 2D gel image evaluation software does not allow exporting designations, the IR1 and IR2 annotations must be assigned manually to the appropriate spots.

id	x	y	comment
1	10	100	IR1
2	12.4	1000	
3	30	500	IR2

Fig 4-5. Correct table format and headings in Excel.

3 Save the file (**File:Save as**) as a Tab delimited text (.txt) file.



id	x	y	comment
1	10	100	IR1
2	12.4	1000	
3	30	500	IR2

Fig 4-6. Correct pick list format and headings.

5 Setting up the Spot Picker

5.1 Select picker head

If the picker head needs to be changed, see instructions in the *Ettan Spot Picker Instrument Handbook*.

5.2 Place the microplates

1 Place the microplate racks in the Spot Picker.

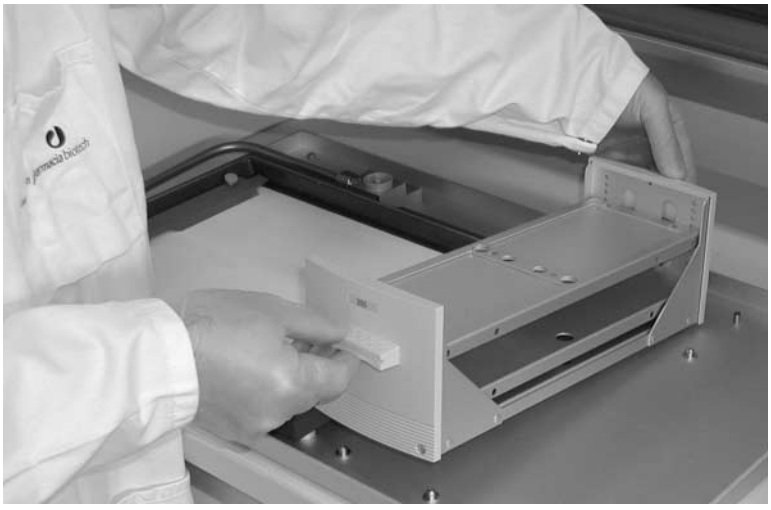


Fig 5-1. Place the microplate racks.

2 Place 1-4 microplates in the plate tray with A1 position in the front.

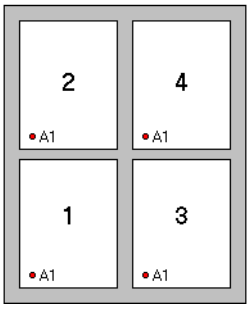


Fig 5-2. Place the microplates.

5.3 Place the gel

1 Place the gel tray in the Ettan Spot Picker. The guide feet on the tray should fit onto pins on the locator plate; it is only possible to fit the gel tray in one direction.

Fig 5-3. Place gel tray.

2 Place the gel in the gel tray and cover with a suitable fluid (e.g. fixing/preservative liquid or double-distilled water) to a surface above the gel of approximately 2 mm. If many spots will be picked, a larger volume of fluid may be required to compensate for evaporation.

Note: *To avoid air bubbles, place fluid in the tray before the gel. The gel is then placed in the tray angled to the buffer surface.*

3 Position the gel so that the reference markers lie within the parallel lines in the centre of the gel tray (see Fig. 5-5). The reference markers must not cross or touch the lines because this will affect the reference marker detection.

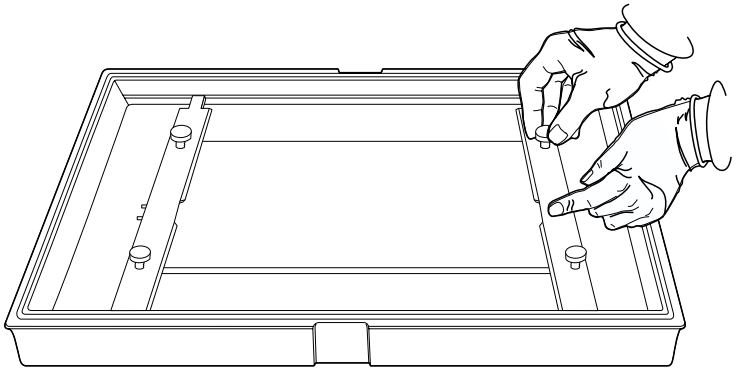


Fig 5-4. Anchor the gel with the gel holders.

4 Assemble the gel holders within the slots in the tray. Anchor the gel down firmly with the gel holders, tightening the screws no more than finger tight.

Note: *For large gels, the gelholders should be positioned in the geltray before the gel is placed. Otherwise, the gel could be damaged by the gel holders.*

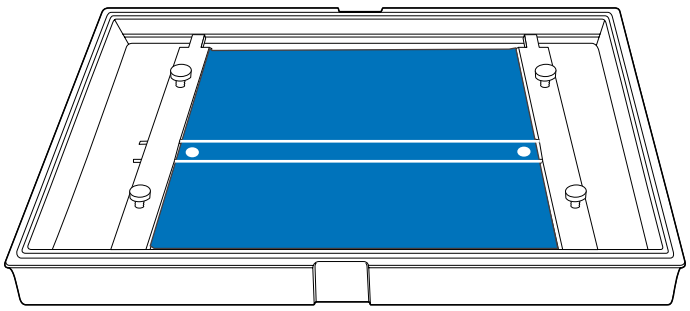


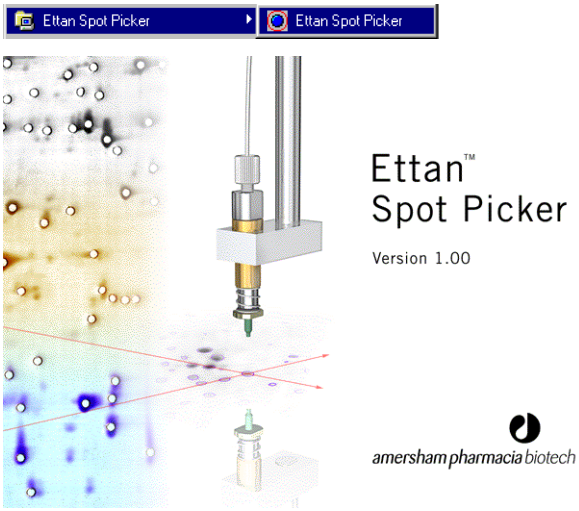
Fig 5-5. Correct position of gel and reference markers in the gel tray.

5.4 Switch on mains power to the instrument

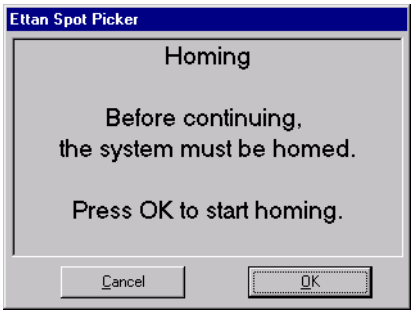
Turn on mains power to the instrument with the mains switch located on the back of the instrument.

5.5 Start Ettan Spot Picker Control software

1 To start the Ettan Spot Picker Instrument Control Software, select **Programs:Ettan Spot Picker:Ettan Spot Picker** from the Windows **Start** menu:



2 A dialogue regarding Homing of instrument will be shown. Press **OK**.



WARNING! MOVING PARTS. The picker head and camera assembly can make sudden, rapid movements. Keep all body parts clear when the Spot Picker is in operation.

5.6 Prime the system

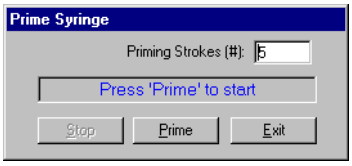
Place the buffer tubing in a suitable buffer for spotpicking. When the picklist is loaded, the software will inform the user of the volume of buffer required to pick all spots. It is recommended that either double-distilled water or the appropriate wash/destain solution for the gel plugs is used as the system buffer.

Priming the syringe removes air bubbles in the spot picker tubing and also rinses the tubing with fresh solution. Priming should be made when tubing, bottles or buffer have been changed.

Note: *It is important to ensure that the position of the rinse station is correctly set in the System Setup (see Ettan Spot Picker Instrument Handbook) before priming the system.*

1 Select **Tools:Prime Syringe.**

2 In the **Prime Syringe** window, enter the number of priming strokes (5-10 priming strokes are recommended). Press **Prime**.



3 The **Stop** button can be used to interrupt the priming.

4 When the priming is completed, press **Exit**.

5.7 System set up

Select **System:System Setup.**

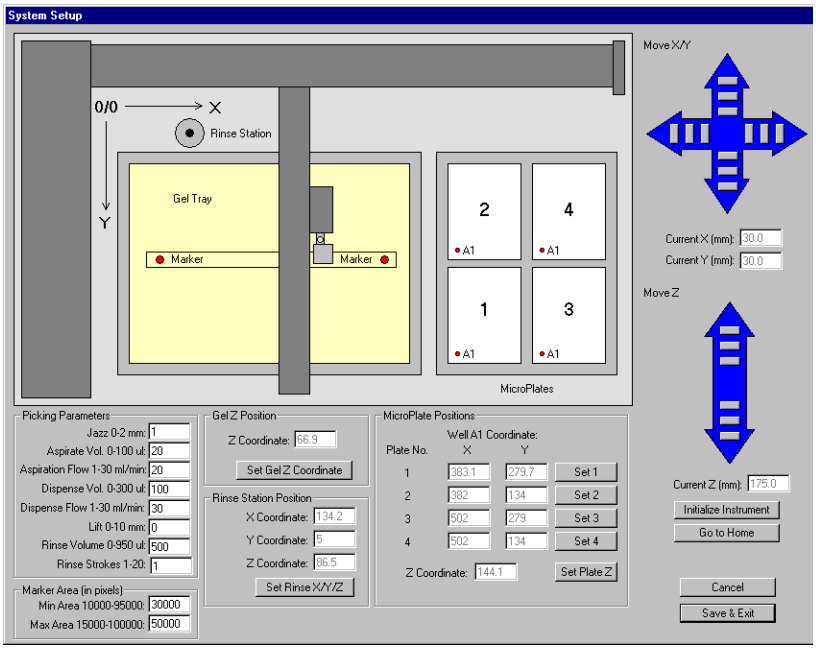


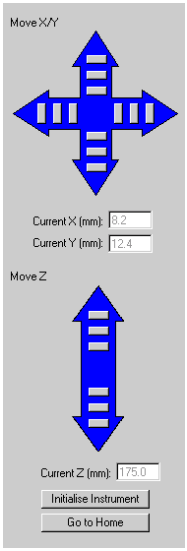
Fig 5-6. The System Set-up window.

The **System Setup** screen allows the user to set:

- The position of the picker head according to microplates and rinse station. For instructions, see the *Ettan Spot Picker Instrument Handbook*.
- The height of the picker head versus the gel backing material.
- The parameters used for picking the gel plugs.
- The reference markers area ranges, which are used by the detection software.

At any stage, the user can decide to abandon the **System Setup** procedure by pressing the **Cancel** button. If the **Cancel** button is pressed, any changes made to the **System Setup** will be lost. To retain new **System Setup** parameters, press the **Save & Exit** button.

It is advisable to press the **Initialize Instrument** and **Go to Home** buttons prior to setting any of the system locations.



The blue arrows visible on the right hand side of the screen are used to manoeuvre the picker head to various positions. Once the picker head is placed appropriately (e.g. in the rinse station), the location can be stored by pressing the appropriate **Set** button.

The blue arrows are used to move the picker head in the X/Y/Z directions. By pressing the buttons on the arrows, the head will move stepwise in the corresponding directions. As long as a button is pressed, the head will keep moving.

For the **Move X/Y** arrow, the outermost buttons will move the head in 10 mm steps, the middle buttons in 1 mm steps and the innermost buttons in 0.1 mm steps.

The **Move Z** arrow buttons will move the head in 5, 1 and 0.1 mm steps, respectively.

CAUTION! Be careful when moving the picker head, because if the head hits an obstacle, the motor will shut-off and the instrument will be re-initialized. Similarly, rapidly moving the picker head into an object has the potential to damage the picker head.

5.7.1 Picker head versus gel Z position

This value indicates the Z-height at which the picker head meets the gel backing. This must be correctly set for maximum picking efficiency.

Note: *This value must be checked before every picking run.*

To set the correct Z-height of the picker head:

1 Use the **Move X/Y** blue arrows to move the picker head to an area where there is gel backing, but no gel, or no important area of the gel.

2 Use the **Move Z** arrows to carefully lower the picker head. Using the innermost button will move the picker head in 0.1mm steps.

3 When the tip of the picker head touches the backing, you will see a gap between the holder and the picker head piston.

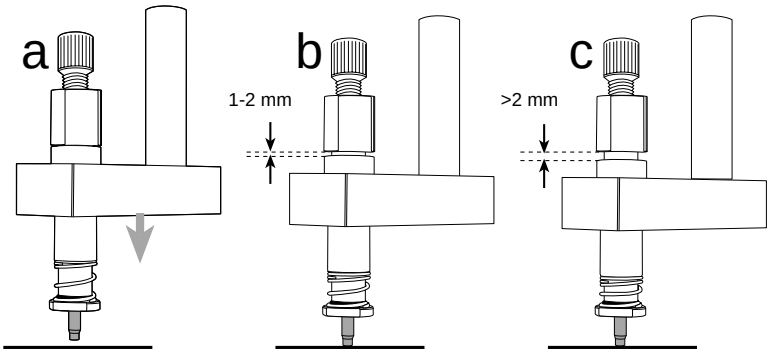


Fig 5-7. Setting the picker head height.
a: Picker head is too high,
b: Picker head is correct height,
c: Picker head is too low.

4 If a gap does not form (Fig. 5-7a), the tip of the picker head is not touching the gel backing. This will result in the gel plugs being left in the gel or picked partially.

If a gap greater than 2 mm can be seen (Fig. 5-7c), the picker head is pressing down too hard on the gel backing. This will result in a shorter life span for the picker head and has the potential to scratch glass plates.

The correct position for the picker head is with a 1-2 mm gap showing at the top of the piston (Fig. 5-7b).

5 Press the **Set Gel-Z Coordinate** when the Z position of the picker head is correct.

5.8 Picking parameters

There are various parameters that must be set correctly for successful picking and dispensing of gel plugs. It is difficult to give exact values for some of them because the optimal values may differ depending on types of backing, percentage gel and staining methods. The parameters can be varied in experiments to optimize the picking from different gel types.

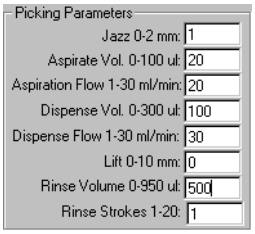


Fig 5-8. Picking parameters

5.8.1 Recommended parameters

Out of performed experiments, some guidelines and recommendations for different type of gels and picker heads have been compiled.

Gel		***Picker head***	***Recommended parameters***					
Thickness	***Backing***	***Type***	***Jazz***	***Asp.vol.***	***Asp. flow***	***Disp.vol.***	***Disp.flow***	***Lift***
1 mm	glass	1.4 x 1.2 2.0 x 1.2	0.9-1.1 1.0-1.2	15-50 30-50	20 20	100-250 150-250	30 30	0 0
	GelBond	1.4 x 1.2 2.0 x 1.2	0.7-1.0 1.1-1.3	15-50 30-50	20 20	100-250 150-250	30 30	0 0
1.5 mm	glass	1.4 x 1.7 2.0 x 1.7	1.2-1.4 1.2-1.4	25-50 25-50	20 20	100-250 150-250	30 30	0 0

Table 5-1. Recommended picking parameters.

5.8.2 Jazz

The **Jazz** parameter determines the amount of sideways movement that the picker head will perform at its lowest Z-position to shear the gel plug from the gel backing. A **Jazz** of 1 mm corresponds to a movement of 1 mm both in the plus and minus direction along the X-axis of the spot picker.

The amplitude of the **Jazz** depends primarily on the picker head size and is also dependent on:

- Type of backing
- Quality of the Bind-Silane treatment
- Percentage of polyacrylamide used
- Gel thickness

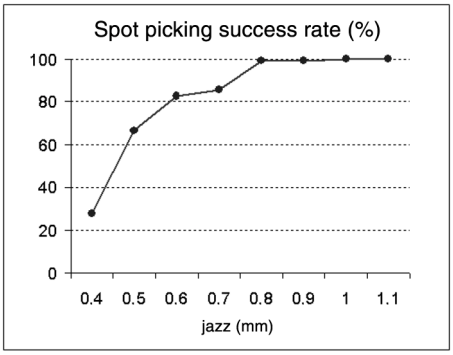


Fig 5-9. Example of effect of jazz amplitude on the picking success rate. A 1.4 x 1.2 picker head was used to pick from 1mm thick gels (12%T) on glass backing.

5.8.3 Aspirate volume and Aspiration flow

The function of aspirating is to suck up the gel plug and keep it in place inside the picker head tip, prior to dispensing into the microplate.

The **Aspirate Vol.** should be large enough to create an adequate negative pressure in the inside of the picker head. Values between 15 and 50 µl have been found to work very effectively. If this value is set too low, then the gel plugs will be left behind in the gel. Values that are too high may lead to some damage of the gel plugs.

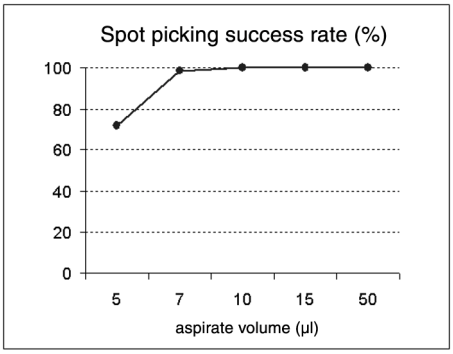


Fig 5-10. Example of effect of aspirate volume on the picking success rate. A 1.4 x 1.2 picker head was used to pick from 1mm thick gels (12%T) on glass backing.

The **Aspiration Flow** determines the rate of aspiration. A value of **20 ml/min** has been shown to work effectively. If this value is set too low, then the gel plugs will be left behind in the gel. Values that are too high may lead to some damage of the gel plugs.

5.8.4 Dispense volume and Dispense flow

Each gel plug is ejected from the picker head along with a volume of fluid. The fluid serves two purposes. Firstly, it physically forces the gel plug out of the picker head. Secondly, if a suitable buffer is used, this can form part of the gel plug washing/destaining procedure.

The **Dispense Vol.** should be large enough to ensure that each gel plug is successfully ejected from the picker head. If this value is set too low, then gel plugs will not be dispensed into the microplate and will be lost when the picker head is next rinsed. The dispense volume should not be below **100** µl.

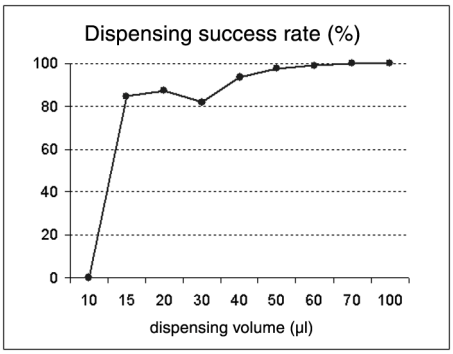


Fig 5-11. Example of effect of dispensing volume on the dispensing success rate. A 1.4 x 1.2 picking head was used to pick from 1mm thick gels (12%T) on glass and GelBond backing.

The **Dispense Flow** needs to be high enough to rapidly eject the plug from the picker head. It is recommended that the dispense flow value is set to max. **30 ml/min.**

5.8.5 Lift

The **Lift** option allows you to raise the picker head from the gel backing prior to aspirating the gel plug into the head. Under normal conditions no lift is required. In some cases introducing a lift may actually reduce the picking efficiency.

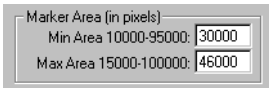
5.8.6 Rinse volume and Rinse strokes

The **Rinse Volume** and **Rinse Strokes** parameters determine the efficiency of picker head washing, after each gel plug is picked. Under normal conditions, a single syringe stroke with a volume 100-950 µl is sufficient. Using more rinse strokes will increase the length of the picking runs.

5.9 Reference Marker area

The reference markers area is set in the **Marker Area** section of the **System Setup** window. The two values (minimum and maximum) stipulate the size (in pixels) of the reference markers. These two values are used during detection of the reference markers to evaluate the objects seen by the camera.

The default values for these parameters are **Min Area:** 30 000 and **Max Area:** 46 000.



5.10 Save and Exit System Setup

Click on **Save & Exit** when the system configuration is completed. The same settings will be used until altered by the user.

6 Picking a gel

6.1 Introduction

For a successful picking run, it is assumed that all the necessary preceding steps have been carried out in accordance with the instructions in this User Manual and *Ettan Spot Picker Instrument Handbook*.

A gel is now placed into the picker and a pick list has been created.

This section describes the actions to perform before, during and after picking:

- Loading of a pick list
- Detection of the reference markers
- Start picking
- Stop/pause picking
- Changing microplates
- Procedure after picking
- Handling of result files

The section also describes the resuming of picking runs:

- When unpicked gel plugs have been located
- When a run has been stopped

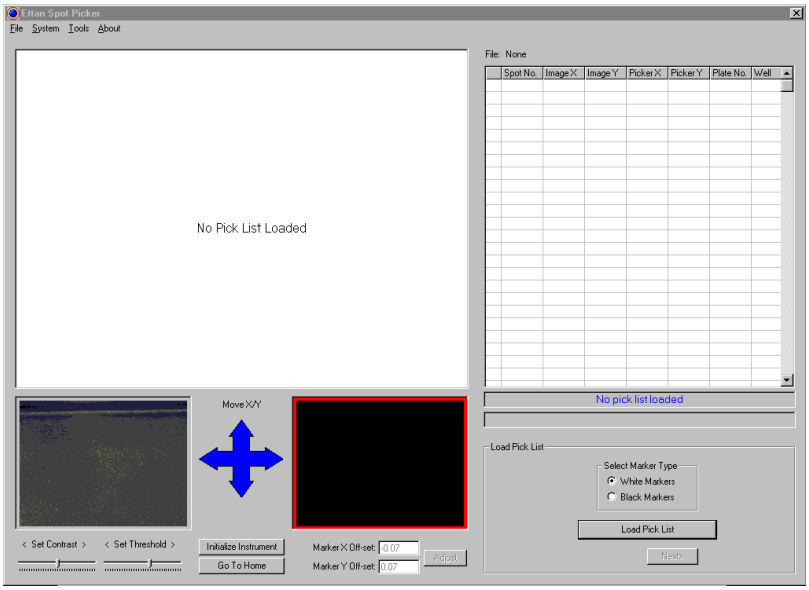
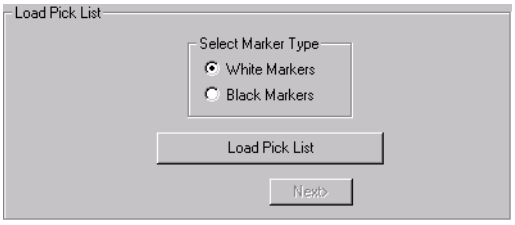


Fig 6-1. Main window before loading a pick list.

Before proceeding, it is advisable to press the **Initialize Instrument** and **Go to Home** buttons.

6.2 Load a pick list

1 In the **Load Pick List** window, select the type of used reference markers (Black/White). Press the **Load Pick List** button.

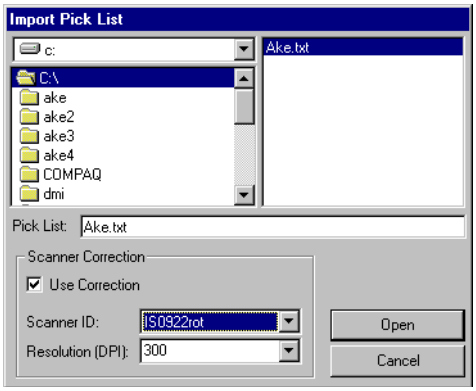


2 Browse for the pick list.

3 Select the pick list.

4 If the gel has been scanned with an ImageScanner, check the **Use Correction** box. Select the used scanner in the **Scanner Id** list box, and select the used resolution when the gel was scanned in the **Resolution (DPI)** list box.

5 Click on **Open.**



6 The pick list data will appear on screen and a representation of the spots to be picked will be drawn in the main part of the window. X and Y coordinate columns are empty, because the reference markers have not been detected yet.

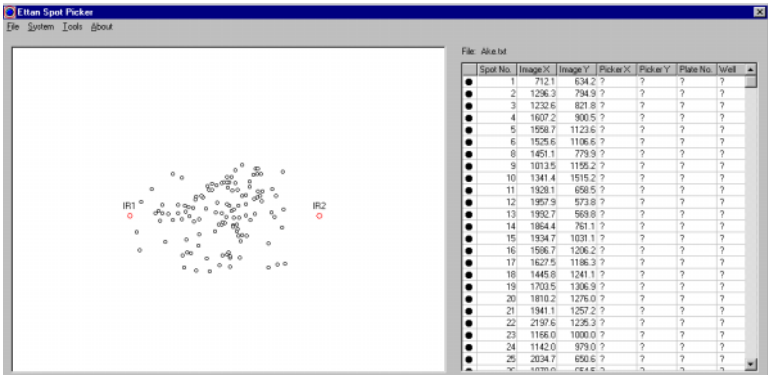
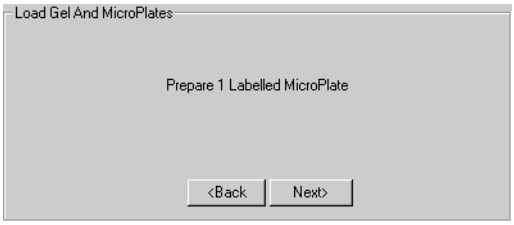


Fig 6-2. Loaded picklist.

7 In the **Load Pick List** window, press **Next.**

8 If microplates have not been previously loaded, label the appropriate number of microplates (as shown on screen) and load the first four (or fewer) onto the Spot Picker microplate rack (See Section 5.2). If a gel has not been loaded, place the gel according to Section 5.3.



9 Press **Next.**

6.3 Detection of the reference markers

1 In the **Find First Marker (IR1)** window, press **Move to First Marker.**

2 Move the camera with the blue arrows to a position over the first reference marker (IR1) on the gel.

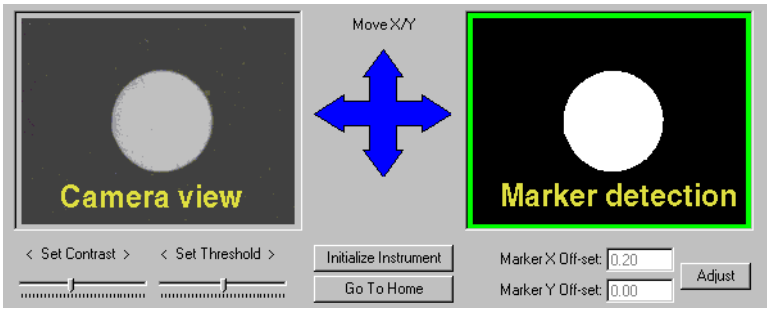


Fig 6-3. Move camera.

3 If the reference marker is underneath the camera, but not visible in the **Camera view** window, use the **Contrast** and **Threshold** sliding bars to find an image of good quality. If the image appears blurry, adjust the camera aperture and focus until the marker image is sharp in the **Camera view.**

4 If the marker is visible in the **Camera view** window, but there is no image in the **Marker detection** window, first adjust the positions of the **Contrast** and **Threshold** sliding bars until an image is visible in the **Marker detection** window. If adjusting the sliding bars does not lead to an image becoming visible in the **Marker detection** window, set the **Contrast** and **Threshold** sliding bars to their central positions. Then

adjust the camera aperture until the marker is visible in the **Marker detection** window.

5 When the majority (or all) of the reference marker is displayed in the **Marker detection** window, the perimeter of the window will change from red to green. This indicates that there is a stable detection of the reference marker. Go to step 9.

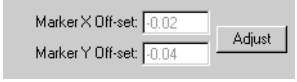
6 If the majority or all of the reference marker is visible in the **Camera view** window, and the perimeter of the **Marker detection** window remains red, then make sure that the **Select Marker Type** box in the **Load Pick List** window has the correct colour of marker selected. Use the **Back** button to go to the **Load Pick List** window. Use the **Next** button to return to the **Find First Marker (IR1)** window. When the perimeter is green, go to step 9.

7 If there is a portion (or none) of the reference marker visible in the **Marker detection** window and the perimeter of the **Marker detection** window is red, this means that the camera is not sufficiently centred over reference marker.

- Adjust the position of the camera using the blue camera movement arrow.
- Return to step **3** and follow the instructions from there onwards until there is a enough of the reference marker visible in the **Marker detection** window to cause the perimeter to turn green.

8 When the perimeter of the **Marker detection** window is green, the Spot Picker has detected a stable image of the reference marker.

9 Below the **Marker detection** window, there are two boxes which show the offset of the centre of the marker from the centre of the camera.



10 Press the **Adjust** button and the reference marker will be centered in the **Marker detection** window. The marker is assumed to be centred when the values in **both** offset boxes are in the range -0.1 to +0.1. The procedure may need to be repeated until the offset values fall within the acceptable range.

Note: *Achieving 0.0 offset values is not required. The software accounts the offset values so that the picking precision will not be affected.*

11 When the offset values are in the range –0.1 to +0.1 press the **Move to First Marker** button.

Note: *It is only when the* **Move to First Marker** *button is pressed that the picker records the position of the marker.*

12 Press **Next**.

13 In the **Find Second Marker (IR2)** window, press **Move to Second Marker**

14 Move the camera with the blue arrows to a position over the second reference marker (IR2) on the gel.

15 When the perimeter of the **Marker detection** window is green, the picker has detected a stable image of the reference marker.

16 If the reference marker is not visible in any of the windows or the perimeter is red, see the step 3-7.

17 Press the **Adjust** button and the reference marker will be centered in the **Marker detection** window. The marker is assumed to be centred when the values in **both** offset boxes are in the range -0.1 to +0.1. The procedure may need to be repeated until the offset values fall within the acceptable range.

18 When the offset values are in the range –0.1 to +0.1, press the **Move to Second marker** button.

19 In the **Find Second Marker (IR2)** window, press **Next**.

The software will now calculate the coordinates for the spots to be picked, and the pick list table on the screen will be updated.

Note: *Damaged reference markers and impurities in the gel image can result in incorrect detections. Also, reflections, shadows and incorrect set contrasts, thresholds and camera aperture can result in similar errors. This will result in imprecise picking.*

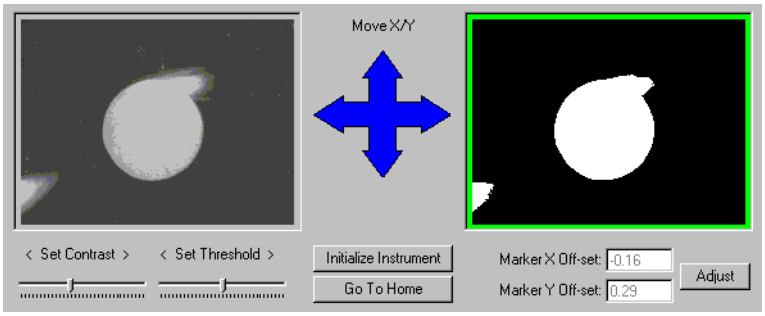


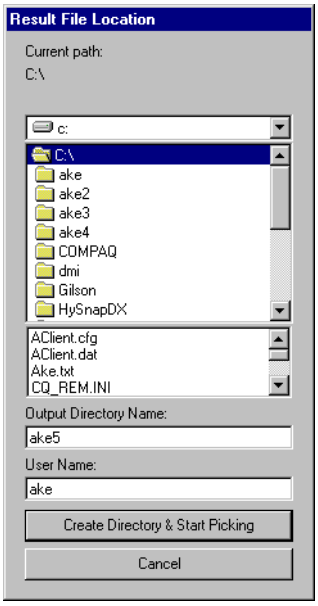
Fig 6-4. Incorrect reference marker detection.

6.4 Pick the gel

1 In the **Pick Spots** window, press **Pick**.

2 In the **Result File Location** window, select the location for the picking results file(s).

3 Enter output directory name and user name. The results will be saved as a folder in the selected location. Each plate filled (even if only partially) during the picking run is given a unique results file.



4 Click on **Create Directory & Start Picking**.

6.5 Pause picking

Press the **Pause** button, if the picking procedure has to be paused to fill the buffer reservoir, for example. When the **Pause** button is pressed, the picker will pause at the end of a specific operation.

Press the **Continue** button to resume picker movement.

6.6 Stop picking

Press the **Stop** button of the instrument, or the **Stop** button in the main application window, if the Spot Picker needs to be immediately stopped.

There is a facility to restart a picking run after pressing **Stop.** This procedure is described in section 6.10.

6.7 Changing microplates

If a picking run consists of more than 384 gel plugs, the software will instruct the user to change plates.

1 Remove the four full plates and cover them with plastic lid or adhesive foil cover.

2 Place the required number of labelled microplates onto the Spot Picker.

3 Press **New Plate**.

This event will happen every time four full microplates are left on the spot picker.

6.8 After picking

When the picking run is completed:

1 Remove the gel from the gel tray and store/discard as appropriate.

2 If the gel is to be discarded, scrape the gel off the glass plate with a plastic spacer or similar object. Then put the plates into a 5% Decon solution overnight to remove any gel fragments which adhere to the plate.

3 Rinse the buffer lines with ddH_20 using the prime syringe tool.

4 Rinse the liquid out of the gel tray with ddH_20 and leave the gel tray to dry.

5 Exit the Ettan Spot Picker Instrument Control Software and turn the computer and the Spot Picker off.

6.9 Repeating a picking run.

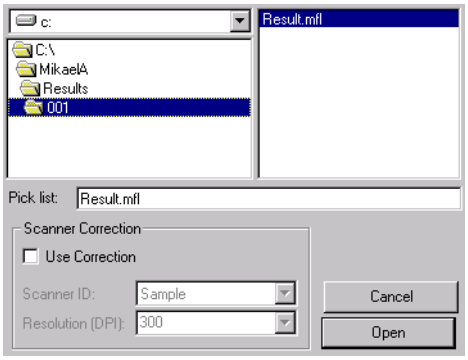
In case of unsuccessfully picked gel plugs, it is possible to repeat a run to complete the picking. The picking parameters may need to be changed, e.g. increase the jazz.

1 Note the positions of microplates and wells with missing gel plugs.

2 In the **Load Pick List** window, press **Load Pick List**.

3 Browse for the result of the performed picking run (extension **.mfl**).

4 Select the result file. Do not use the correction function again! Click on **Open**.



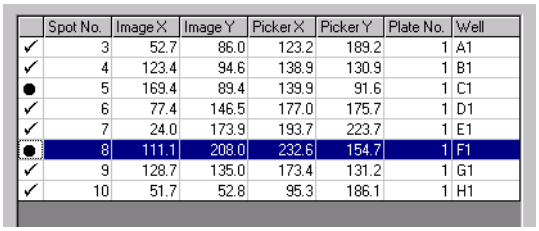
5 The result table will be shown on the screen.

	Spot No.	Image X	Image Y	Picker X	Picker Y	Plate No.	Well
✓	3	52.7	86.0	?	?	?	?
✓	4	123.4	94.6	?	?	?	?
✓	5	169.4	89.4	?	?	?	?
✓	6	77.4	146.5	?	?	?	?
✓	7	24.0	173.9	?	?	?	?
✓	8	111.1	208.0	?	?	?	?
✓	9	128.7	135.0	?	?	?	?
✓	10	51.7	52.8	?	?	?	?

Note: *The detection of reference markers has to be done again. The Picker X and Y values are missing in the table.*

6 Perform detection of reference marker, see Section 6.3

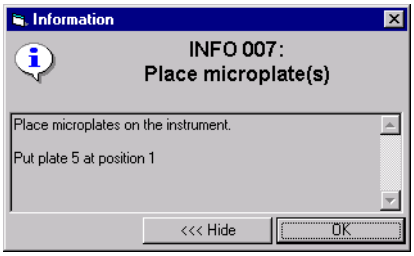
7 In the updated result table, select the rows for each unpicked gel plug and the ticks in the table will be replaced by filled circles.



	Spot No.	Image X	Image Y	Picker X	Picker Y	Plate No.	Well
✓	3	52.7	86.0	123.2	189.2	1	A1
✓	4	123.4	94.6	138.9	130.9	1	B1
●	5	169.4	89.4	139.9	91.6	1	C1
✓	6	77.4	146.5	177.0	175.7	1	D1
✓	7	24.0	173.9	193.7	223.7	1	E1
●	8	111.1	208.0	232.6	154.7	1	F1
✓	9	128.7	135.0	173.4	131.2	1	G1
✓	10	51.7	52.8	95.3	186.1	1	H1

8 Press **Pick**. The user will be informed by the software to place the specific plate in position 1.

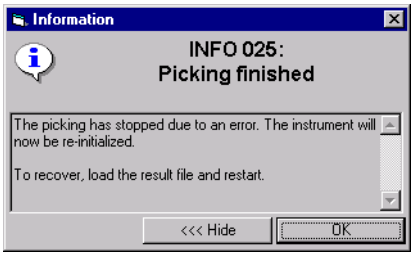
Note: *It is important to remove the volumes of liquid in the empty wells otherwise the volume will be too high after re-picking.*



9 Press **OK** and the picking run will start.

6.10 Resume picking after Ettan Spot Picker has been stopped

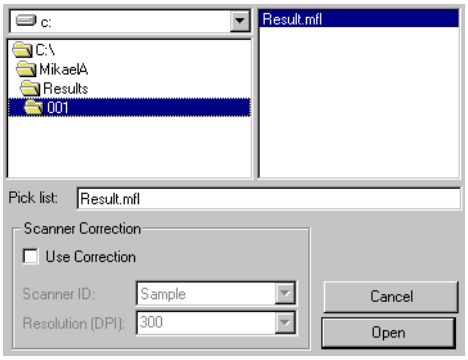
1 When the Ettan Spot Picker has been stopped by user or due to a technical error, an information message appears. Press **OK**.



2 In the **Load Pick List** window, press **Load Pick List**.

3 Browse for the result of the interrupted picking run (extension **.mfl**).

4 Select the result file. Do not use the correction function again! Click on **Open**.



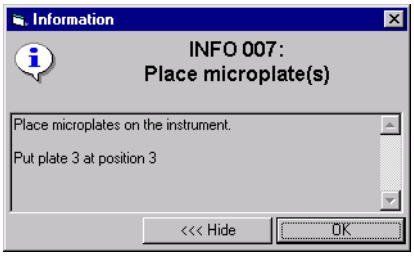
5 The result table will be shown on the screen. Ticked positions mean picked gel plugs. Filled circles represent unpicked gel plugs.

	Spot No.	Image X	Image Y	Picker X	Picker Y	Plate No.	Well
✓	3	46.9	146.2	?	?	?	?
✓	4	46.9	292.3	?	?	?	?
✓	5	46.9	438.5	?	?	?	?
●	6	46.9	584.6	?	?	?	?
●	7	46.9	730.8	?	?	?	?
●	8	46.9	876.9	?	?	?	?
●	9	46.9	1023.1	?	?	?	?
●	10	46.9	1169.2	?	?	?	?
●	11	46.9	1315.4	?	?	?	?

Note: *The detection of reference markers has to be done again. The Picker X and Y values are missing in the table.*

6 Perform detection of reference marker, see Section 6.3.

7 Press **Pick**. The user will be informed by the software to place the microplates. The partially dispensed micro plate and the next undispensed microplates must be placed in their original positions.



Note: *Depending on exactly when picking was interrupted, the last gel plug being picked may be lost.*

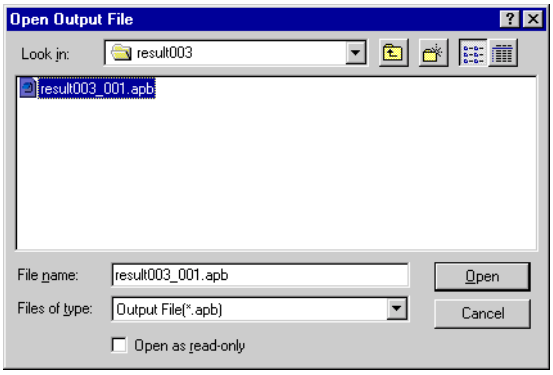
6.11 Open result files

The format for the file names is Directory name_XX.apb, where the directory name is the same as the name entered in the **Output Directory Name** field and the XX is the number of the plate produced in that picking run.

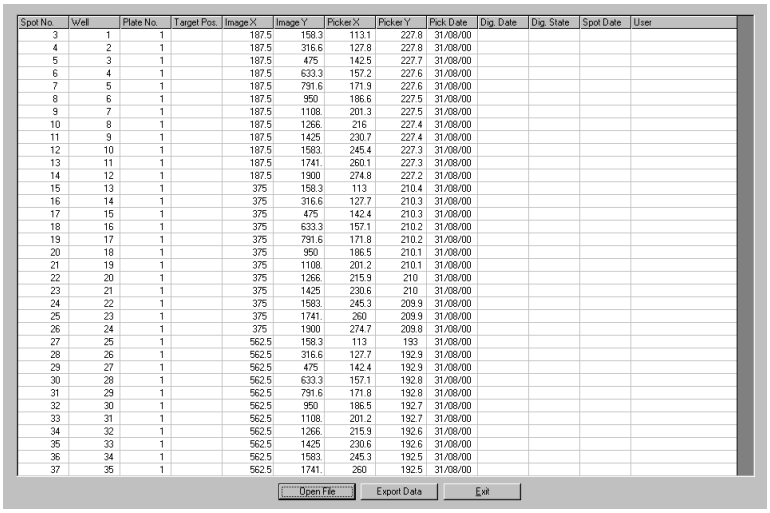
1 To view result files associated with each microplate of gel plugs, select **Tools/Output File Viewer.**

2 To open a plate file, press the **Open File** button in the lower end of the dialogue.

3 Browse and select the file to be viewed and click **Open**.



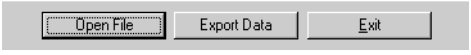
4 The viewer allows the user to look at the processing status of the gel plugs in that micro plate.



Spot No.	Well	Plate No.	Target Pos.	Image X	Image Y	Picker X	Picker Y	Pick Date	Dig. Date	Dig. State	Spot Date	User
3	1	1		187.5	158.3	113.1	227.8	31/08/00				
4	2	1		187.5	316.6	127.8	227.8	31/08/00				
5	3	1		187.5	475	142.5	227.7	31/08/00				
6	4	1		187.5	633.3	157.2	227.6	31/08/00				
7	5	1		187.5	791.6	171.9	227.6	31/08/00				
8	6	1		187.5	950	186.6	227.5	31/08/00				
9	7	1		187.5	1108.	201.3	227.5	31/08/00				
10	8	1		187.5	1266.	216	227.4	31/08/00				
11	9	1		187.5	1425.	230.7	227.4	31/08/00				
12	10	1		187.5	1583.	245.4	227.3	31/08/00				
13	11	1		187.5	1741.	260.1	227.3	31/08/00				
14	12	1		187.5	1900	274.8	227.2	31/08/00				
15	13	1		375	158.3	113	210.4	31/08/00				
16	14	1		375	316.6	127.7	210.3	31/08/00				
17	15	1		375	475	142.4	210.3	31/08/00				
18	16	1		375	633.3	157.1	210.2	31/08/00				
19	17	1		375	791.6	171.8	210.2	31/08/00				
20	18	1		375	950	186.5	210.1	31/08/00				
21	19	1		375	1108.	201.2	210.1	31/08/00				
22	20	1		375	1266.	215.9	210	31/08/00				
23	21	1		375	1425.	230.6	210	31/08/00				
24	22	1		375	1583.	245.3	209.9	31/08/00				
25	23	1		375	1741.	260	209.9	31/08/00				
26	24	1		375	1900	274.7	209.8	31/08/00				
27	25	1		562.5	158.3	113	193	31/08/00				
28	26	1		562.5	316.6	127.7	192.9	31/08/00				
29	27	1		562.5	475	142.4	192.9	31/08/00				
30	28	1		562.5	633.3	157.1	192.8	31/08/00				
31	29	1		562.5	791.6	171.8	192.8	31/08/00				
32	30	1		562.5	950	186.5	192.7	31/08/00				
33	31	1		562.5	1108.	201.2	192.7	31/08/00				
34	32	1		562.5	1266.	215.9	192.6	31/08/00				
35	33	1		562.5	1425.	230.6	192.6	31/08/00				
36	34	1		562.5	1583.	245.3	192.5	31/08/00				
37	35	1		562.5	1741.	260	192.5	31/08/00				

Open File | Export Data | Exit

5 To export data, press the **Export Data** button.



6 Save the data as a text file which may then be used in other applications such as Microsoft Word™ and Microsoft Excel.

7 Picking a gel without a pick list

7.1 Introduction

The Ettan Spot Picker allows spot picking in a mode similar to manually picking the spots. This feature is useful, when there are a limited number of spots to pick. The gel does not need to be scanned, and no pick list must be prepared.

The picking will not produce a result file, so the user must keep track of the spots.

Note: *Use the delivered white sheet as a background to obtain a better contrast of the gel image in the camera view.*

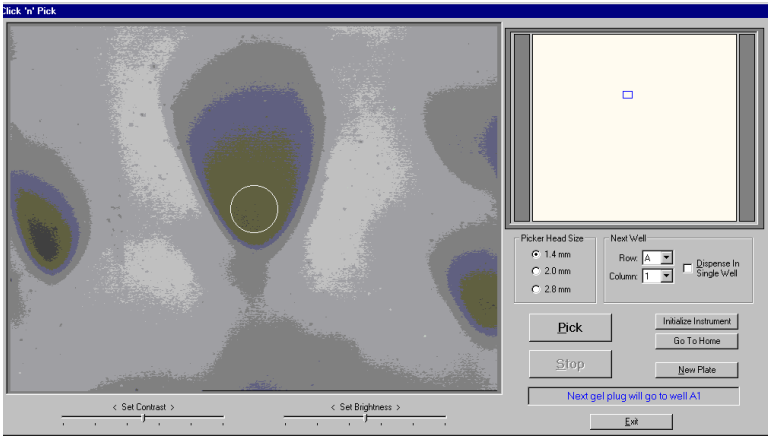
7.2 Manual picking directly from gel without a pick list

The Ettan Spot Picker, gel and microplates must have been prepared according to Chapter 5 Setting up the Spot Picker.

The function uses the picking parameters as set in the System setup. Therefore, these need to be set correctly before using Click n'Pick.

1 Press **Initialise Instrument** and **Go to Home.**

2 Select **Tools:Click n'Pick.**



3 Select the **Picker Head Size**. This will alter the size of the circle in the camera view window.

4 The first gel plug will be dispensed in microplate well A1. However, the position can be edited in the **Next Well** window

5 Set **Contrast**, **Brightness** and camera aperture, to achieve a good image quality.

6 Move the square in the upper right window with the mouse pointer to a preferred position of the gel.

7 Move the circle in the camera view by using the mouse pointer. When a requested gel spot is detected within the picker circle, press **Pick**.

Tip! It may be convenient to place e.g. a pen close to the spot which will help to find it in the camera view. Do not activate the movements of the picker during this procedure!

Note: *When the picker head has dispensed the gel plug into the micro plate, the camera will move back to the picked position.*

WARNING! MOVING PARTS. The picker head and camera assembly can make sudden, rapid movements. Keep all body parts clear when the Spot Picker is in operation.

8 If several gel plugs from a spot are wanted to be pooled in the same microplate well, it is possible by checking the **Dispense in Single Well** box in the **Next Well** window. This has to be done before the first picking to that well.

9 When the microplate is full. Replace the plate with an empty plate, and press **New Plate**.

10 When the picking run is completed. Press **Exit**.

8 Troubleshooting

To avoid errors and to obtain maximum picking success rate, it is important to follow all the instructions in the User Manual and Instrument Handbook.

The user must ensure that:

- The picker head is not damaged, and is clean on both the inner and outer sides.
- The O-ring is present, and not damaged.
- Tubing is clean, and not damaged.
- The Z-height values are correctly set.
- The System setup parameters are correctly set.

Problem	***Probable cause***	***Remedy***
Gel plugs not removed from gel.	The set jazz amplitude is too low.	Increase the jazz value stepwise with 0.1 mm increments.
	Inefficient aspiration.	The aspiration volume should be minimum 15 µl. Increase the aspiration volume. Check the O-ring of the picker head for damage. Check all tubing for leaks and air bubbles.
	Bind-Silane treatment was too effective.	Reduce the concentration of Bind-Silane.
	The picker head does not reach the gel.	Check the picker head's Z-height versus gel.
	The picker head is worn-out.	Replace the picker head.
	A position, close to the current gel plug's, has previously been picked.	

Problem	*Probable cause*	*Remedy*
Gel plugs are picked but deformed or not complete.	Only the upper part is picked due to the picker head not reaching the gel backing.	Adjust the picker head versus gel Z-position.
	The inner side of the picker head is dirty.	Clean the picker head (both inner and outer sides).
Gel from spot has been picked but is not found in microplate well.	Gel plug is situated in a nearby well. The microplate Z-height has been too high so the dispense flow has splashed it into a nearby well.	Decrease the microplate Z-height.
	Gel plug is in rinse station due to adsorption to the outer side of the picker head during dispensing. The dispensing height has been set too low.	Increase the microplate Z-height. Clean the outer side of the picker head to reduce liquid adsorption.
	Gel plug is in rinse station due to adsorption to the outer side of the picker head during dispensing. The dispensing volume has been set too low.	Increase the dispensing volume. Clean the outer side of the picker head to reduce liquid adsorption.
	Gel plug is lost during transfer from gel to microplate.	Increase the aspiration volume.
Very imprecise picking results.	The identities of the two reference markers have been mixed up.	Confirm that the first detected marker is the correct one on the gel image.

Problem	*Probable cause*	*Remedy*
Picking position errors.	Camera is not correctly calibrated.	Check that the calibration has been performed according to the Instrument Handbook.
	Incorrect detection of the reference markers.	Be sure to have a distinct circular picture of the marker both in the camera and marker detection view.
	Uncorrected ImageScanner image.	Scan according to User Manual and use the correction option when the pick list is imported.
	There is no calibration file for the used scanner.	Contact APBiotech Service for calibration of the scanner.
	The gel image has too low resolution.	Scan the gel with higher resolution, for example 300 dpi.
Picking errors for oval and irregular shaped spots	The centre of the detected spot in the 2D-software differs from the preferred picking position.	Detect these types of spots manually. For example with the Grow Peaks tool or the Pen tool.
Scratches on the gel glass plates after picking.	The Z-height of the picker head versus gel is too low.	Adjust the Z-height according to User Manual.
The gel loosens from the glass plate.	Inefficient Bind-Silane treatment.	Prepare according to recommendations in User Manual. Clean the plates properly and leave them in 5% Decon over night.
The gel loosens from the glass plate in a specific area during picking.	Too many gel plugs have been picked from a smaller area.	
The gel cracks and become damaged close to the picked gel plug.	Gel stored in ethanol and acetic acid has a tendency to crack. This will not affect the picking result, if not too many spots are picked from the same area.	Storage of the gel in double distilled water reduces the risk of cracking.

9 Staining protocols

9.1 Gel fixing

When the gel run is complete, the gel must be placed into a suitable fixing solution as soon as possible. This will minimize protein spot diffusion. As the gel is attached to a backing, a fix solution that dehydrates the gel too quickly will cause the gel to crack and peel away from the backing.

It is recommended that a solution of 30% ethanol and 7.5% acetic (ethanoic) acid is used to fix gels. The gels must be left in this fix solution for at least two hours. Leaving gels in this fix for longer periods of time appears to have no detrimental effects on the gel.

9.2 SYPRO ruby

1 Fix the gel in 10% methanol and 7% acetic acid for at least 2 hours.

2 Place the gel directly into a polypropylene, polycarbonate or polyvinyl chloride tray.

3 Cover the gel with the SYPRO ruby stain.

4 Incubate the gel for five hours - overnight with gentle shaking, protected from the light.

5 Pour off the SYPRO ruby.

6 Wash the gel in 10% methanol, 6% acetic acid for 1-2 hours.

9.3 SYPRO orange

1 Place the gel directly into a polypropylene, polycarbonate or polyvinyl chloride tray.

2 Prepare 250 ml of a 1:5000 dilution of SYPRO orange in 7.5% acetic acid.

3 Stain the gel for three hours - overnight with gentle shaking, protected from the light.

4 Wash the gel in 7.5% acetic acid for 60-90 minutes.

9.4 Silver staining compatible with MS analysis

9.4.1 Using the Modified PlusOne™ method

Allow at least 250 ml of each solution per gel.

All steps are carried out at room temperature with gentle shaking.

Make all solutions freshly when needed. Make sure to use only double distilled (18.2 MΩ) water. All solutions should appear clear and colourless before they are poured onto the gel.

Reagent	*Quantity*
Ethanol	75 ml
5% Sodium thiosulphate solution	10 ml
Sodium acetate	17 g
ddH_20	To a final volume of 250 ml

Table 9-1. Sensitizing solution.

Reagent	*Quantity*
Silver nitrate	625 mg
ddH_20	To a final volume of 250 ml

Table 9-2. Silver nitrate solution (0.25%).

Reagent	*Quantity*
Sodium carbonate	6.25 g
Formaldehyde	100 µl
ddH_20	To a final volume of 250 ml

Table 9-3. Developing solution.

Reagent	*Quantity*
EDTA	3.65 g
ddH_20	To a final volume of 250 ml

Table 9-4. Stop solution.

1 Sensitize the gel in Sensitizing solution for 30 min.

2 Rinse the gel three times in ddH_2O, for five minutes each rinse.

3 Incubate in a 0.25% silver nitrate solution for 20 minutes.

4 Rinse the gel twice in ddH_2O, for five minutes each rinse.

5 Incubate the gel in Developing solution for up to 10 minutes. If the staining is intense enough before the end of 10 minutes, move directly to the stop solution.

6 Pour the developing solution off and add the Stop solution, incubate for 10 minutes.

7 Rinse the gel three times in ddH_2O, for five minutes each rinse.

8 Store the gel in ddH_2O.

9.4.2 Using the Hoefer automatic gel stainer

The following method was developed for 1 mm thick 12% acrylamide gels. Other thickness and percentage acrylamide gels may require further optimization. The wash steps which follow the sensitizing and silver stages are crucial for a low background to the gel.

Make all solutions freshly when needed. Make sure to use only double distilled (18.2 MΩ) water. All solutions should appear clear and colourless before they are poured onto the gel.

Reagent	*Quantity*
Sodium thiosulphate	500 mg
ddH_20	To a final volume of 250 ml

Table 9-5. Sodium thiosulphate solution (0.2%).

Reagent	*Quantity*
Glacial acetic (ethanoic) acid	12.5 ml
ddH_20	To a final volume of 250 ml

Table 9-6. Stop solution.

1 Fix the gel for 2 hours minimum in 30% ethanol, 7.5% acetic acid.

2 Wash the gel four times in 50% methanol, for eight minutes each rinse.

3 Sensitize for 30 minutes in 0.2% sodium thiosulphate.

4 Wash the gel five times in ddH_2O, for 20 minutes each rinse.

5 Incubate in 0.25% silver nitrate solution (Table 9-2) for 30 minutes.

6 Wash the gel twice in ddH_2O, for four minutes each rinse.

7 Incubate in developing solution (Table 9-3) for 2-5 minutes.

8 Incubate in Stop solution (Table 9-6) for 60 minutes.

9 Store the gel in ddH_2O.

9.5 Coomassie staining

1 Fix the gel for 60 minutes in 30% ethanol and 7.5% acetic acid.

2 Stain the gel for 60 minutes minimum in 0.1% Coomassie R-250 in 40% ethanol and 10% acetic acid. Use a Stock solution of 1 w/vol.% Coomassie R-250 in 96% ethanol.

3 Destain the gel in 20% ethanol and 5% acetic acid.

4 Store the gel in ddH_2O.

Index

M

P

R

S

T